BATCH SOLVENT EXTRACTION OF CAFFEINE FROM *MCBC2*

DINESH VARMA A/L NEDUNJELIYAN

A thesis submitted in fulfillment
of the requirements for the award of the Degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical and Natural Resources Engineering
University Malaysia Pahang

September 2010

ABSTRACT

Caffeine is a naturally occurring substance found in cocoa seeds. The aim of this research is to extract caffeine from *Malaysian Cocoa Board Clone (MCBC) 2*, and to investigate the effect of sample particle size, solvent/feed ratio, and extraction time on the yield of caffeine. The sample was prepared by grinding and sieving, followed by solid-liquid extraction using water by heat reflux extracting technique, liquid-liquid extraction with ethyl acetate, drying of caffeine by rotary evaporator, and finally analysis of the caffeine yield. The analysis of the caffeine yield was done using UV/Vis Spectrophotometric method. The caffeine yield was highest at sample particle size of 400µm (0.35 % w/w caffeine or 3.4956 mg/g cocoa), solvent/feed ratio of 1:1 (0.35 % w/w caffeine or 3.5066 mg/g cocoa), and extraction time of 90 minutes (0.34 % w/w caffeine or 3.4356 mg/g cocoa). The best conditions for the highest yield of caffeine from *MCBC2* were 400µm of sample particle size, 1:1 of solvent/feed ratio, and 90 minutes of extraction time.

ABSTRAK

Kafein adalah sebuah zat yang dijumpai secara semulajadi dalam biji koko. Objektif kajian ini adalah untuk mengekstrak kafein dari *Klon Lembaga Koko Malaysia (MCBC) 2*, dan untuk mengkaji pengaruh saiz zarah koko, nisbah pelarut/sampel, dan masa ekstraksi terhadap hasil kafein. Persiapan sampel dilakukan dengan mengisar dan menapis, diikuti oleh ekstraksi pepejal-cair menggunakan air panas dengan teknik ekstraksi refluks, diikuti ekstraksi cair-cair dengan pelarut etil asetat, pengeringan kafein dengan rotary evaporator, dan akhirnya analisis hasil kafein. Analisis hasil kafein dilakukan dengan menggunakan kaedah spektrofotometri UV/Vis. Hasil kafein yang tertinggi diperolehi pada saiz zarah sampel 400µm (0.35 % w/w kafein atau 3.4956 mg/g koko), nisbah pelarut/sampel 1:1 (0.35 % w/w kafein atau 3.5066 mg/g koko), dan masa ekstraksi 90 minit (0.34 % w/w kafein atau 3.4356 mg/g koko). Keadaan terbaik untuk mendapatkan hasil tertinggi kafein dari *MCBC2* adalah pada saiz zarah sampel 400µm, nisbah pelarut/sampel 1:1, dan masa ekstraksi 90 minit.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
MCBC2	Malaysian Cocoa Board Clone 2
SPE	Solid Phase Extraction
UV	Ultraviolet
UV/Vis	Ultraviolet/Visible

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Since a decade ago, Malaysia was recognized as the largest cocoa producer, and Malaysia is ranked 11th in the list of cocoa cultivating countries, worldwide (Anonym., 2005). Commonly, the alkaloid contents (caffeine, theobromine, and theophylline) in cocoa are extracted before the cocoa is processed, and the alkaloids, especially caffeine are discarded without use. Actually, according to some research, caffeine has its own benefits, for example it is used for pharmaceutical and therapeutic purposes. Malaysia as a large producer of cocoa, can extract the caffeine in the cocoa, and process the caffeine for benefits, without discarding it. Therefore, an efficient and economic method of extraction of caffeine from cocoa is needed to get a high yield of caffeine. Moreover, an effective and low cost solvent is also needed for better extraction of caffeine. This will increase the profit from the sales of caffeine.

Caffeine is a naturally occurring substance found in the leaves, seeds or fruits of more than 63 plants species worldwide. The most common sources of caffeine are coffee, cocoa beans, cola nuts, tea leaves, yerba mate, guarana berries, and the Yaupon Holly. Caffeine is the most widely consumed psychoactive substance and can be a mild central nervous system stimulant. It does not accumulate in the body over a period of time and is normally excreted within several hours of consumption (Barone and Roberts, 1996).

Caffeine is an alkaloid of the methylxanthine family, thus it is known as 1,3,7-trimethylxanthine. Caffeine is an intensely bitter white powder in its pure state. Its IUPAC name is 1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione, with chemical formula $C_8H_{10}N_4O_2$ (Arnaud, 1987). The structure of caffeine is shown in Figure 1.1, below (Mumin *et al*., 2006).

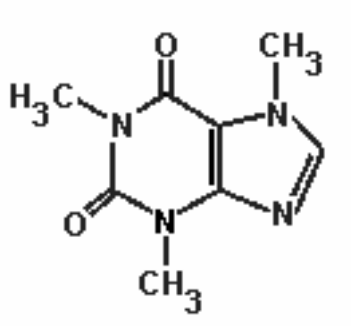


Figure 1.1: The structure of caffeine

Cocoa tree is an evergreen tree in the family *Sterculiaceae*, genus *Theobroma*, and species *cacao*, which flourish well in a narrow belt of 10^o of either sides of the Equator. Climatically, Malaysia is very suitable for cocoa growing, since cocoa tree grow well in humid tropical climates with regular rains and a short dry season. There are three broad types of cocoa; *Forastero, Crillo, and Trinitario* which is a hybrid of *Forastero* and *Crillo* (Anonym., 2010). Cocoa is an important flavouring ingredient in preparation of beverages, confectionary, ice-cream, bakery products, etc. The stimulant action of cocoa based products is due to the presence of alkaloids, theobromine, and caffeine in them. Theobromine accounts for about 90% of the total composition of cocoa, while the remaining is caffeine (Franzke *et al*., 1969).

One analysis of the chemical composition of cocoa beans after fermentation and drying is shown in Table 1.1, below:

Table 1.1: Chemical composition of cocoa beans (Minifie, 1989)

Contents	**Nib % Maximum**	**Shell % Maximum**
Water	3.2	6.6
Fat (cocoa butter, shell fat)	57	5.9
Ash	4.2	20.7
Total nitrogen	2.5	3.2
Theobromine	1.3	0.9
Caffeine	0.7	0.3
Starch	9	5.2
Crude fibre	3.2	19.2

This indication of the chemical composition of cocoa beans can vary depending on the type of bean, the quality of the fermentation and drying, and the subsequent processing of the bean.

Caffeine can be extracted from cocoa by various methods, such as water extraction, supercritical carbon dioxide extraction, and organic solvent extraction. Solvents such as chloroform, methyl chloride, ethanol, and ethyl acetate are commonly used for the solvent extraction of caffeine (Anonym., 2010). Several methods can be used for this extraction purpose, for example Soxhlet extraction, Ultrasonic extraction, and Heat Reflux extraction. The Heat Reflux extraction is one of the common methods used to extract caffeine from cocoa seed on a laboratory scale.

The heat reflux extractor is shown in Figure 1.2, below (Anonym., 2010).

Figure 1.2: Heat reflux extractor

Malaysian Cocoa Board Clone 2 (*MCBC2*) cocoa is a new local breed of cocoa, which was cloned by the Malaysian Cocoa Board. This breed is planted in Jengka, Pahang. The picture of this *MCBC2* tree is shown in Appendix A. Cocoa tree is subject to attack by a large number of pests and diseases, where the most important group of pests are the capsids, and the most universal cocoa disease is caused by the fungus *Phytophthora palmivora* (Anonym., 2010). The *MCBC2* cocoa breed is modified so that it could stand the attacks by these kinds of pests and diseases. Since this breed is a new breed growing in Jengka, Pahang, the composition of caffeine in this cocoa seeds is yet to be analyzed. Therefore, this research may benefit the Malaysian Cocoa Board in analyzing their new breed.

CHAPTER 2

LITERATURE REVIEW

2.1 Cocoa

Cocoa was domesticated by the Mayas and Aztecs thousands of years ago. Cocoa has travelled along the trade routes used by the Mayas, Aztecs, and also the Pipil-Nicaraoseven before the Spanish conquest (Sophie and Michael, 1996). In the year 1525, Criollo types of cocoa have spreaded to Central America, and to a large number of Caribbean islands, including Trinidad, and Jamaica. Then, cocoa was introduced into Central America, particularly Costa Rica, by the Spanish people. Around the year 1750, the French people planted cocoa in Martinique and Haiti, and the Portuguese people planted cocoa in Belem and Bahia, using Lower Amazon (Forastero) type of cocoa.

In the 18th Century, between Criollo and Forastero types of cocoa was hybridized and Trinitario types of cocoa was founded (Pittier, 1933). According to Pound (1945), the two populations could have met and hybridised on the islands of the Orinoco delta, including Trinidad and the Orinoco valley. Cheesman (1944) reported that, in the year 1727, the ‘Blast”, which is a cyclone or an epidemic has destroyed the Criollo plantations in Trinidad. Then, the cocoa plantations were reconstituted using Trinitario seeds from the Orinoco valley. This Trinitario hybrid of cocoa was produced by open pollination. Their superiority in agronomic terms and better resistance to diseases and pests has favoured their use in Trinidad as a replacement for Criollo types of cocoa.

Young (1994) stated that, in the 16th century, cocoa was introduced into Asia and the Pacific. In 1560, the Dutch introduced the Venezuelan Criollo trees into Java, Indonesia. Meanwhile, in the year 1614, the Spanish introduced Criollo types of cocoa into the Philippines from Mexico. Cocoa was taken by the British to Madras, India from the island of Amboina in the year 1798, and it was introduced into Sri Lanka from Trinidad at about the same time. From Sri Lanka, cocoa was transferred to Singapore and Fiji in year 1880, Samoa in year 1883, Queensland in year 1886, and Bombay in year 1887.

In Malaysia, the first cocoa was planted in Malacca in the year 1778. Subsequently, the cocoa planting was started in area at Serdang Agriculture Station and Silam Agriculture Research Center, Sabah. The earliest cocoa commercialization was started between the years 1853 to 1959 where Amelonado cocoa types were first planted at Jerangau, Terengganu. The planted area was about 403 hectarages. Cocoa trial was further undertaken at Serdang, Cheras, Kuala Lipis and Temerloh between the years 1936 to 1940. However, cocoa was only actively planted after the World War II, whereby cocoa officially came to Quoin Hill, Tawau, Sabah in the year 1960. From then on, cocoa has become an important commodity in Malaysian economy.

2.1.1 Scientific Classification of Cocoa

Cocoa tree is originated from the Kingdom *Plantae*, Subkingdom *Tracheobionta*, Division *Magnoliophyta*, Class *Magnoliopsida*, Subclass *Dilleniidae*, Order *Malvales*, Family *Sterculiaceae*, Genus *Theobroma L.*, and Species *Theobroma cacao L.* (Anonym., 2010).

2.1.2 Characteristics of Cocoa Tree

The cocoa tree is a tropical plant that grows in hot, rainy climates. The cultivation of cocoa is concentrated on a narrow band of no more than 20 degrees north or south of the Equator. Cocoa trees need rainfall between 1,150 and 2,500 millimeters per year without hot dry winds and drought, and an even temperature between 21°C and 32°C for ideal growth (Anonym., 2010).

The cocoa tree is usually a small tree of 4 to 8 meters tall. Its stem is straight, the wood is light and white, and the bark is thin, and brownish in colour. The leavesof the cocoa tree are alternate, entire, unlobed, 10 to 40 centimeters long, and 5 to 20 centimeters broad. The leaves are poisonous and inedible as they are filled with a creamy and milky liquid, which tastes spicy and unpleasant.Cocoa trees begin to bear fruit when they are 3 to 4 years old.The cocoa fruit (pods) can reach up to 15 to 25 centimeters in length, 8 to 10 centimeters in wide, and weighs about 500 grams when ripe. Each pod contains about 20 to 40 seeds, which are known as cocoa beans after drying and fermentation. The seeds are in reddish-brown colour externally and are covered by a white, sweet pulp. Each cocoa tree will yield 20 to 30 pods per year and the peak times for harvesting are around the months April and September in Malaysia (Anonym., 2010).

2.1.3 Types of Cocoa

There are three broad types of cocoa Forastero and Crillo plus Trinitario which is a hybrid of Forastero and Crillo. Within these types are several varieties, Forastero, which now forms the greater part of all cocoa grown, is hardy and vigorous producing beans with the strongest flavour. Amelonado is the Forastero variety most widely grown in West Africa and Brazil. It has a smooth yellow pod with 30 or more pale to deep purple beans (Anonym., 2010).

Crillo with its mild or weak chocolate flavour is grown in Indonesia, Central and South America. Crillo trees are not as hardy and they produce softer pods which are red in colour, containing 20-30 white, ivory or very pale purple beans. Trinitario plants are not found in the wild as they are cultivated hybrids of the other two types. Trinitario cocoa trees are grown mainly in the Caribbean area but also in Cameroon and Papua New Guinea. The mostly hard pods are variable in colour and they contain 30 or more beans of variable colour but white beans are rare (Anonym., 2010).

2.2 Caffeine

2.2.1 Properties

Pure caffeine occurs as odourless, white, fleecy masses, glistening needles of powder. Its molecular weight is 194.19 g/gmol, melting point is 236 ^{o}C, point at which caffeine sublimes is 178 ^{o}C, at atmospheric pressure, pH is 6.9 (1% solution), specific gravity is 1.2, volatility is 0.5 %, vapour pressure is 760 mm Hg at 178 ^{o}C, solubility in water is 2.17 g per 100 mL water at 25 ^{o}C, and vapour density is 6.7 (Clementz and Dailey, 1988).

The pure caffeine was first isolated by a German chemist Friedrich Ferdinand Runge in year 1819 (Weinberg and Bealer, 2001). The nitrogen atoms in the structure of caffeine are all planar (in sp^2 orbital hybridization), resulting in the aromatic characteristics of caffeine. Caffeine is a readily available by-product of decaffeination, and it is not usually synthesized (Anonym., 2001). But if desired, caffeine can be synthesized from dimethylurea and malonic acid (Wilson and Norman, 2004).

2.2.2 Applications

Caffeine is the world's most widely consumed psychoactive substance, by which the global consumption of caffeine has been estimated at 120,000 tonnes per year (Anonym., 1997). Caffeine can be a mild central nervous system stimulant, depending on its dose. Caffeine does not accumulate in the body over the course of time and is normally excreted within several hours of consumption. Caffeine is a central nervous system and metabolic stimulant (Nehlig *et al.*, 1992), and it is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs. Caffeine and other methylxanthine derivatives are also used on newborns to treat apnea (suspension of external breathing) and treat irregular heartbeats. Caffeine also stimulates the central nervous system first at the higher levels, resulting in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination, and later at the spinal cord level at higher doses (Bolton and Null, 1981).

According to Leo (1992), caffeine which is found in cocoa, tea, and coffee imparts bitterness and also acts as a flavour constituent. It is a mild nervous stimulant towards drowsiness and fatigue, thus it is used by athletes to enhance performance since it mobilizes fats from stores a process that normally does not become maximal until intense activity is underway (Eva, 1988). Caffeine is used as a drug on the basis of its effect on the respiratory, cardiovascular and the central nervous system. Caffeine is included with aspirin in some preparations for treatment of headaches as it decreases cerebral eye blood flow. Caffeine is also included with ergotamine in some antimigraine preparations, in order to produce a mildly agreeable sense of alertness (Lawrence, 1986).

According to Jeanne (1987), caffeine is administered in the treatment of mild respiratory depression which caused by central nervous system depressants such as narcotic. Caffeine is also used in the treatment of acute circulatory failure. It is also used to relieve fatigue in either beverage or in non-prescription tablet form, since it increases the amount of urine flow. There are about 2000 non-prescription and about 1000 prescription drugs containing caffeine (Jeanne, 1987).

2.2.3 Disadvantages

Consumption of caffeine in large amounts, and especially over extended periods of time, can lead to a condition known as caffeinism (Mackay and Rollins, 1989). Caffeinism usually combines caffeine dependency with a wide range of unpleasant physical and mental conditions including nervousness, irritability, anxiety, tremulousness, muscle twitching (hyperreflexia), insomnia, headaches, respiratory alkalosis, and heart palpitations (Leson *et al.*, 1988). It also increases the production of stomach acid, thus high usage over time can lead to peptic ulcers, erosive esophagitis, and gastroesophageal reflux disease (Anonym., 2009). Caffeine also stimulates the stomach to pour out large amounts of acid. This in turn leads to burning in the pits of the stomach and aggravates peptic ulcers of the stomach and duodenum. It also may induce benign (non cancerous) breast diseases and may worsen premenstrual symptoms in women who overuse it. Caffeine crosses the placenta and enters the fetal circulation and its use at a pharmacological level has been associated with low birth weight. Excessive consumption during lactation may cause irritability and wakefulness in a breast- fed baby (Eva, 1988).

2.3 Extraction of Caffeine

Decaffeination is a popular term in present modern world to optimize the caffeine contents in various sources. This is simply use of a solvent, which extract caffeine. For this purpose, the currently available solvents are chloroform, methyl chloride, ethyl acetate, super critical carbon dioxide etc.

The industrial decaffeination process has evolved over the years. Initially, direct contact methods used chloroform ($CHCl_3$), and more recently methylene chloride (CH_2Cl_2), as the solvent to repeatedly rinse the green (unroasted) cocoa beans that had been softened by steam. Once sufficient caffeine had been removed, the beans would be roasted. Since these organic solvents have a high vapour pressure and low boiling point, any solvent remaining in the beans is removed during roasting. This method has several brown characteristics. Both of these solvents are carcinogenic and have several human health concerns with methylene chloride having the lesser overall hazard. Chlorinated hydrocarbon waste has significant environmental impacts and is costly to dispose. Roasting also does not guarantee full removal of the solvent, although solvent levels are rarely detectable. Although these solvents have its disadvantages, they are still used because they are not water-soluble, have a low boiling point, and remove caffeine without removing significant amounts of other compounds, leaving the majority of the flavour unaltered (Kirmer, 1988).

Recently the direct contact process has been greened significantly using supercritical CO_2. The green cocoa beans are steam softened with water and then supercritical CO_2 is used to extract the caffeine. Once the system is returned to room temperature and pressure the cocoa beans and separated caffeine are now solvent free as CO_2 returns to the gas phase. Then the CO_2 can be captured and reused. This method has all the advantages of the above technique without the environmental and human health risks (Murray, 1995).

Indirect contact methods have also been developed to decaffeinate cocoa. The green cocoa beans are soaked (steeped) in almost boiling water until the caffeine is removed from the bean. The cocoa solution is then treated with ethyl acetate (a natural ester) which has moderate human health hazards but is not carcinogenic. Ethyl acetate solvates caffeine more effectively than water and extracts the caffeine. The remaining ethyl acetate is removed from the cocoa solution by steaming. The cocoa solution is then combined with the beans which reabsorb the cocoa oils as they are dried. 2-Propanol is also used as extraction solvent rather than ethyl acetate as it is less hazardous to human health (Hampp, 1996).

2.3.1 Types of Solvent

The isolation of caffeine from cocoa is known as decaffeination, which is done by using a solvent that extract the caffeine. For this purpose, the common solvents used are chloroform, methyl chloride, ethyl acetate, super critical carbon dioxide, etc. Methylene chloride is also used to extract caffeine from cocoa, and it is highly effective, but methylene chloride is potentially dangerous under certain circumstances. It can cause faintness, dizziness, and headache if inhaled at high concentrations (Kirmer, 1988). Ethyl acetate is another compound used to extract caffeine from cocoa. It removes caffeine from cocoa effectively, and it extracts other chemical components from the cocoa as well. Ethyl acetate is much less hazardous to health and environment compared to chlorinated solvents (Johnson *et al.*, 1988). Water, although an excellent solvent of methylxanthines, but it is highly non-selective and its use may result in the removal of other valuable components from the extracted product, which gradually leads to deterioration of the analytical column (Saldana *et al*., 2002).

2.3.2 Methods of Extraction of Caffeine

In a research done by Hu *et al*. (1997), caffeine was extracted from tea using ethanol solvent, by heat reflux extraction. A 50% ethanol in water was refluxed at 85^{o}C, for 45 minutes. The extract was then filtered through a filter paper, and the filtered solution was centrifuged for 10 minutes, at a speed of 4000rpm. The supernatant was then analyzed to determine the caffeine composition.

Hu *et al*. (1997) has also done a research of extracting caffeine from tea using ultrasonic extraction method. 50% ethanol in water was used as solvent to extract the caffeine from tea, and the solution was sonicated for 90 minutes in an ultrasonic bath (frequency 50Hz, power 250W) at 20-40^{o}C. Then the extract was filtered, and the filtered solution was centrifuged for 10 minutes, at a speed of 4000rpm. The supernatant collected was the analyzed to know the caffeine composition.

Ramli *et al*. (2000) has analyzed the total polyphenols, epicatechin, catechin, theobromine and caffeine contents in Commercial cocoa and chocolate products such as cocoa powder, cocoa beans, cocoa liquor and chocolate using High Performance Liquid Chromatography (HPLC). The methylxanthines were identified and quantified using Bondapak column and mobile phase of methanol:water:acetic acid at ratio 20:79:1. 32 samples of chocolate products were analyzed, and the levels of caffein and theobromine were 0.62-1.14 mg/g and 0.026-0.153 mg/g, respectively. The chocolate coating made from fat substitute had theobromine and caffeine levels ranged from 0.36-0.70 mg/g and 0.027-0.061 mg/g, respectively. The mean theobromine and caffeine levels in local chocolates respectively were 0.72 mg/g and 0.04mg/g in milk chocolate, and 0.85 mg/g and 0.06 mg/g in dark chocolate. In imported chocolates, the mean theobromine and caffeine levels respectively were 1.05 mg/g and 0.12 mg/g in dark chocolate, 0.76 mg/g and 0.04 mg/g in milk chocolate, and 0.74 mg/g and 0.03 mg/g in white chocolate. The imported chocolates have higher level of theobromine and caffeine compared with the local chocolates.

Mumin *et al*. (2006) has done a research on determination and characterization of caffeine in tea, coffee, and soft drinks by Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC). Caffeine which is a mild addicting drug was isolated, purified and characterized from tea (black and green) and coffee. The isolation of caffeine was done by liquid-liquid extraction using chloroform as the extracting solvent. Four steps of extraction were carried out such as leaching, dye removal, liquid extraction and recrystallization. Toluene and petroleum ether were the solvent used for recrystallization. The crude caffeine was purified by SPE method. For the characterization of pure caffeine by HPLC, 50mM KH_2PO_4 (pH=2), acetonitrile, and methanol at ratio 40:8:2 was used as solvent as well as mobile phase at ratio. The amount of caffeine in various soft drinks (Cola) that commercially available in Bangladesh were also determined by HPLC method.

Abourashed and Mossa (2004) have done HPTLC determination of caffeine in stimulant herbal products and power drinks. They analyzed the caffeine content in selected herbal products and energy drinks available in the Saudi market by HPTLC–UV densitometric. Pre-coated HPTLC silica gel plates (20 cm × 10 cm), and a solvent system consisted of ethyl acetate–methanol (85:15, v/v), and caffeine were used for the analysis, at 275 nm. The levels of caffeine in the herbal products and the energy drinks were 4.76–13.29% (w/w) and 0.011–0.032% (w/v), respectively.

Li *et al.* (1989) have developed a method for the determination of theobromine and caffeine in cocoa beans using UV spectrophotometer. They have presented a rapid, simple and accurate method for individually determining theobromine and caffeine in cocoa beans. Caffeine alone was completely extracted into chloroform from an aqueous solution at a pH between 12.5 and 12.7, and analyzed by UV spectrophotometer at 275.9nm. For the remaining theobromine in the aqueous solution, a wavelength of 272.7nm was used. A result with relative standard deviation of about 0.65% was obtained.

In a study done by Wanyika *et al.* (2010), the levels of caffeine in certain coffee (nescafe, africafe, dormans) and tea (chai mara moja, kericho gold, sasini, finlays premium) brands were determined using high performance liquid chromatography (HPLC) and UV/Vis Spectrophotometric methods. The levels of caffeine in all the tea and coffee brands were found to be within the documented range. Generally, higher concentration of caffeine in all the samples were realized with the UV/Vis Spectrophotometric method compared to HPLC method. This indicates that acidified water was a better caffeine extractor than pure water. The results showed that the levels of caffeine obtained by UV/Vis Spectrophotometric method were much higher than those obtained by HPLC method. This shows that acidified water is a more efficient extractor of caffeine.

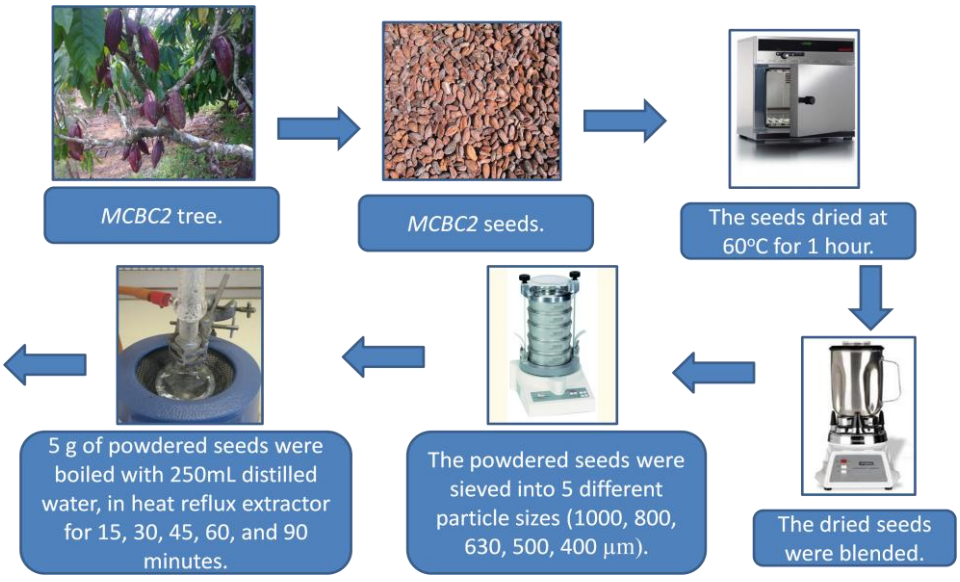
CHAPTER 3

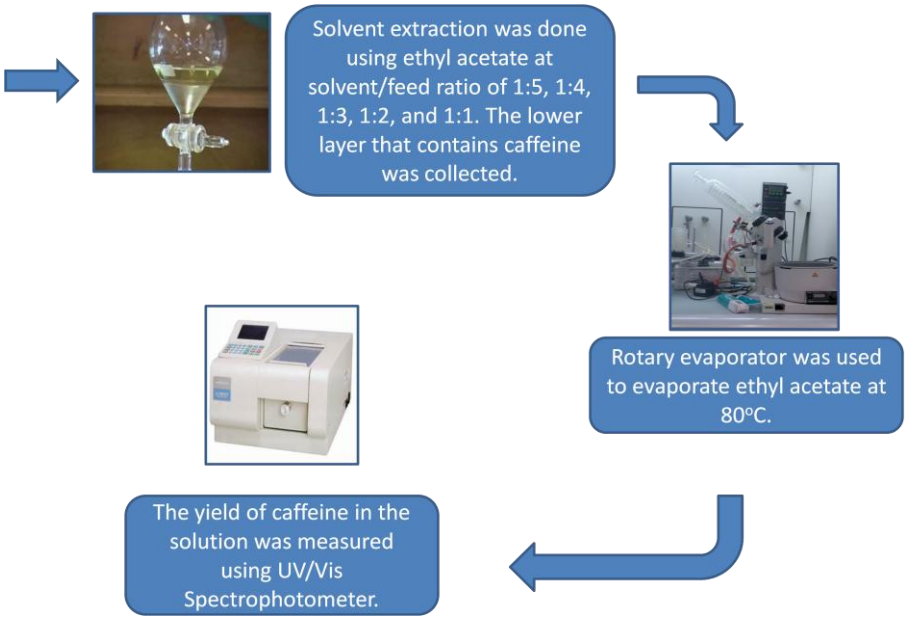
METHODOLOGY

3.1 Materials

The *MCBC2* cocoa seeds were bought from Malaysian Cocoa Board research station in Jengka, Pahang. Three parameters were set up to investigate its effect on the caffeine yield. The equipments and apparatus used in this research were beaker, heater, vacuum filter, separatory funnel, pH meter, rotary evaporator, electronic balance, oven, heat reflux extractor, Buchner funnel, exicator, and siever. The chemicals and reagents used in this research were distilled water, 10% lead ethanoate solution, ethyl acetate, solid sodium hydrogen carbonate, 1M sodium hydroxide solution, and anhydrous sodium sulphate.

3.2 Flowchart





3.3 Methods

3.3.1 Preparation of Sample

50 grams of the *MCBC2* cocoa seeds were weighed and dried in incubator at 60^{o}C for 2 hours, to remove the moisture in the seeds. Then, the seeds were blended to get powdered sample. Next, the powdered sample was sieved into 5 different particle sizes, which are 1000, 800, 630, 500, and 400 µm.

3.3.2 Preparation of Solutions

10% (w/v) lead acetate solution was prepared by adding 10 grams of anhydrous lead acetate into 100 mL of distilled water. 1M sodium hydroxide solution was prepared by adding 4 grams of anhydrous sodium hydroxide into 100 mL of distilled water.